

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of
Takumi TERATANI et al. : Group Art Unit: 1633
Serial No. 10/591,407 : Examiner: Quang NGUYEN
Filed: December 8, 2006 :
For: RAT EMBRYONIC STEM

DECLARATION UNDER 37 CFR 1.132

Commissioner for Patents
P.O. Box 1450
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Sir:

I, Takahiro Ochiya, declare:

That I am a citizen of Japan, and my post office address
is National Cancer Center Research Institute, 5-1-1,
Tsukiji, Chuo-ku, Tokyo 104-0045, Japan;

That my education and employment history is as shown in my
Curriculum Vitae attached hereto (Attachment 1):

That I am a co-inventor of the above-identified U.S.
patent application SN 10/591,407 and directed and
supervised the following experiment, which was carried out
to determine the effect of rat leukemia inhibitory factor
(rLIF) on the efficiency of formation of rat inner cell
masses from rat blastocysts, the results of which follow
hereunder;

EXPERIMENTS

[Materials & Methods]

(1) Oocyte Sampling

A female WKY/N (Wistar Kyoto strain, Charles River
Laboratories Japan, 10-week-old or older) was naturally
crossbred, at 3 days after vaginal plug confirmation, the

female rat for oocyte sampling was sacrificed and the uterus was excised. The embryo was recovered and developed to become blastocyst (late stage) in 5% CO₂ incubator.

(2) Preparation of Feeder Cell

As a feeder cell to be used for the establishment and culture of rat ES cell, normal fibroblast of fetal mouse ICR at 12.5 days treated with mitomycin C was used (FIG. 2). Before use, a cryopreserved feeder cell was thawed one day before use, and cultured using STO medium (DMEM 450 ml, FBS 50 ml, Antibiotic-Antimicrototics solution 5 ml) and a gelatin-coated culture dish (Iwaki, Tokyo, Japan).

(3) Consideration of LIF Addition

The necessity of addition of a rat leukemia inhibitory factor (rLIF) to a culture medium during the step of formation of an inner cell mass from rat blastocyst was considered. Various concentrations (0-5000 units) of rLIF (Chemicon) were added during the step of formation of an inner cell mass from rat blastocyst, and the blastocyst was cultured for 7 days. The number of the inner cell masses formed was observed.

[Results]

As a result, inner cell masses were efficiently formed in the absence of LIF (30%), rather than in the presence of various concentrations of rLIF (0-16.7%) as shown below.

rat LIF (U)	No. of rat blastocysts	No. of rat inner cells masses formed
5000	6	0
2500	6	0
1000	6	1
500	6	0
100	6	1
0	10	3

That I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Signed this 11 day of November, 2009.



Takahiro OCHIYA

Curriculum Vitae

November 11, 2009

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1988 Osaka University, Graduate School of Medicine (Ph.D. awarded)

Professional History:

1988-1992 Research Assistant, Institute for Molecular and Cellular Biology, Osaka
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1993-1998 Section Head, Genetics Division, National Cancer Center Research Institute
1998- Section for Studies on Metastasis, National Cancer Center Research Institute
1991-1992 The Barnham Institute Medical Research, La Jolla, CA USA
2004- Visiting Professor, Waseda University, Graduate School of Science and
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2008- Visiting Professor, Tokyo Institute of Technology School and Graduate
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Society: Japan Society of Gene Therapy
Japanese Cancer Association
Japanese Biochemical Society
The Molecular biology Society of Japan

Publication

1. Adenovirus-mediated transfer of the HST-1(FGF4) gene induces increased levels of platelet count in vivo.
Proceedings of the National Academy of Sciences USA (Sakamoto H, Ochiya T, Sato Y, Tsukamoto M, Konishi H, Saito I, Sugimura T, Terada M.) vol.91 No.26, 12368-12372 1994
2. Adenovirus-mediated transfer of HST-1/FGF-4 gene protects mice from lethal irradiation.
Oncogene (Y. Takahama, T. Ochiya, H. Tanooka, H. Yamamoto, H. Sakamoto, Hiroshige Nakano and Masaaki Terada.) vol.18 No.43, 5943-5947 1999
3. New delivery system for plasmid DNA in vivo using atelocollagen as a carrier material: the Minipellet.
Nature Medicine (T. Ochiya, Y. Takahama, S. Nagahara, Y. Sumita, A. Hisada, H. Itoh, Y. Nagai and M. Terada.) vol.6, 707-710 1999

4. PDGF alpha-receptor is unresponsive to PDGF-AA in aortic smooth muscle cells from the NG2 knockout mouse.
J Cell Sci. (K. A. Grako, T. Ochiya, D. Barritt, A. Nishiyama and W. B. Stallcup.) vol.112, 905-915 1999
5. Detection of spatial localization of Hst-1/Fgf-4 gene expression in brain and testis from adult mice.
Oncogene (Hanako Yamamoto, Takahiro Ochiya, Yasushi Takahama, Yasuo Ishii, Noriko Osumi, Hiromi Sakamoto and Masaaki Terada.) vol.19, 3805-3810 2000
6. Mouse flt-1 promoter directs endothelial-specific expression in the embryoid body model of embryogenesis.
Biochem Biophys Res Commun. (G. Quinn, T. Ochiya, M. Terada, and T. Yoshida.) vol.276 No.3, 1089-1099 2000
7. Biomaterials for Gene Delivery: Atelocollagen-mediated Controlled Release of Molecular Medicines.
Current Gene Therapy (Takahiro Ochiya, Shunji Nagahara, Akihiko Sano, Hiroshi Itoh, and Masaaki Terada.) vol.1 No.1, 31-52 2001
8. High susceptibility of transgenic rats carrying the human c-Ha-ras proto-oncogene to chemically-induced mammary carcinogenesis.
Mutation Research (Tsuda H, Asamoto M, Ochiya T, Toriyama-Baba H, Naito A, Ota T, Sekiya T, Terada M.) vol.477, 173-182 2001
9. Involvement of poly(ADP-ribose) polymerase in trophoblastic cell differentiation during tumorigenesis.
Mutation Research (Masutami M, Nozaki T, Watanabe M, Ochiya T, Hasegawa F, Nakagama H, Suzuki H, Sugimura T.) vol.477 No.1-2, 111-117 2001
10. Atelocollagen-based gene transfer in cells allows high-throughput screening of gene functions.
Biochem Biophys Res Commun (Honma K, Ochiya T, Nagahara S, Sano A, Yamamoto H, Hirai K, Aso Y, Terada M.) vol.289 No.5, 1075-1081 2001
11. HST-1/FGF-4 gene activation induces spermatogenesis and prevents adriamycin-induced testicular toxicity.
Oncogene (Yamamoto H, Ochiya T, Tamamushi S, Toriyama-Baba H, Takahama Y, Hirai K, Sasaki H, Sakamoto H, Saito I, Iwamoto T, Kakizoe T, Terada M.) vol.21 No.6, 899-908 2002
12. Enhanced skin carcinogenesis in Cyclin D1-conditional Transgenic mice.
Cancer Research (Yamamoto H, Ochiya T, Takeshita F, Toriyama-Baba H, Hirai K, Sasaki H, Sasaki H, Sakamoto H, Yoshida T, Saito I, Terada M.) vol.62 No.6, 1641-1647 2002
13. Mammalian Rcd 1 is a novel transcriptional cofactor that mediates retinoic acid-induced cell differentiation.
EMBO Journal (Hiro N, Ito T, Yamamoto H, Ochiya T, Jinno S, Okayama H.) vol.21 No.19, 5235-5244 2002
14. Differentiation of embryonic stem cells into hepatocytes: biological functions and therapeutic application.
Hepatology (Yamamoto H, Quinn G, Asan A, Yamanokuchi H, Taratani T, Terada M, Ochiya T.) vol.37 No.5, 983-993 2003

15. Atelocollagen-mediated delivery of synthetic small interfering RNA for effective gene silencing in vitro and in vivo.
Nucleic Acids Res. (Minakuchi Y, Takeshita F, Kosaka N, Sasaki H, Yamamoto T, Kouno M, Honma K, Nagahara S, Hanai K, Sano A, Kato T, Terada M, Ochiya T.) vol.32, e109 2004
16. HST-1/FGF-4 plays critical role in crypt cell survival and facilitates epithelial cell restitution and proliferation.
Oncogene (Sasaki H, Hirai K, Yamamoto Y, Tanooka H, Sakamoto H, Iwamoto T, Takahashi T, Terada M, Ochiya T.) vol.23, 3681-3688 2004
17. Direct hepatic fate specification from mouse embryonic stem cells.
Hepatology (Teratani T, Yamamoto H, Aoyagi K, Sasaki H, Asari A, Quinn G, Sasaki H, Terada M, Ochiya T.) vol.41 No.4, 836-846 2005
18. Efficient delivery of small interfering RNA to bone-metastatic tumors by using atelocollagen in vivo.
Proceedings of the National Academy of Sciences USA (Takeshita F, Minakuchi Y, Nagahara S, Honma K, Sasaki H, Hirai K, Teratani T, Namatame N, Yamamoto Y, Hanai K, Kato T, Sano A, Ochiya T.) vol.102 No.34, 12177-12182 2005
19. Recapitulation of in vivo gene expression during hepatic differentiation from murine embryonic stem cells.
Hepatology (Yamamoto Y, Teratani T, Yamamoto H, Quinn G, Murata S, Ikeda R, Kinoshita K, Matsubara K, Kato T, Ochiya T.) vol.42 No.3, 558-567 2005
20. MOZ is essential for maintenance of hematopoietic stem cells.
Genes and Development (Katsumoto T, Aikawa Y, Iwama A, Ueda S, Ichikawa H, Ochiya T, Kitabayashi I.) vol.20 No.10, 1321-1330 2006
21. FGF-4 regulates neural progenitor cell proliferation and neuronal differentiation.
FASEB Journal (Kosaka N, Kodama M, Sasaki H, Yamamoto Y, Takeshita F, Takahama Y, Sakamoto H, Kato T, Terada M, Ochiya T) vol.20 No.9, 1484-1485 2006
22. Hedgehog signal activation in gastric pit cell and in diffuse-type gastric cancer.
Gastroenterology (Fukaya M, Isohata N, Ohta H, Aoyagi K, Ochiya T, Saeki N, Yanagihara K, Nakanishi Y, Taniguchi H, Sakamoto H, Shimoda T, Nimura Y, Yoshida T, Sasaki H.) vol.131 No.1, 14-29 2006
23. A photon counting technique for quantitatively evaluating progression of peritoneal tumor dissemination.
Cancer Research (Yanagihara K, Takigahira M, Takeshita F, Komatsu T, Nishio K, Hasegawa F, Ochiya T.) vol.66 No.15, 7532-7539 2006
24. Adipose tissue-derived mesenchymal stem cells as a source of human hepatocytes.
Hepatology (Banas A, Teratani T, Yamamoto Y, Tokuhara M, Takeshita F, Quinn G, Okochi H, Ochiya T.) vol.46 No.1, 219-228 2007
25. Stem cell plasticity: learning from hepatogenic differentiation strategies.
Developmental Dynamics (Banas A, Yamamoto Y, Teratani T, Ochiya T.) vol.236 No.12, 3228-3241 2007
26. Atelocollagen for protein and gene delivery.(Review)
Adv Drug Deliv Rev. (Sano A, Maeda M, Nagahara S, Ochiya T, Honma K, Itoh H, Miyata T, Fujioka K.) vol.55 No.12, 1651-1677 2003

27. The role of atelocollagen-based cell transfection array in high-throughput screening of gene functions and in drug discovery.(Review)
Curr Drug Discov Technol. (Honma K, Miyata T, Ochiya T.) vol.1 No.4, 287-294 2004
28. Therapeutic potential of RNA interference against cancer.(Review)
Cancer Science (Takeshita F, Ochiya T.) vol.97 No.8, 689-696 2006
29. "Stem cells into liver"--basic research and potential clinical applications.(Review)
Adv Exp Med Biol. (Banas A, Quinn G, Yamamoto Y, Teratani T, Ochiya T.) vol.585, 3-17 2006
30. Application of atelocollagen-mediated siRNA delivery for RNAi therapies.(Review)
Yakugaku Zasshi (Honma K, Takeshita F, Ochiya T.) vol.127 No.5, 807-812 2007
31. Atelocollagen-mediated drug discovery technology.
Expert Opin Drug Discov. (Ochiya T, Honma K, Takeshita F, Nagahara S.) vol.2, 159-167 2007
32. Establishment of rat embryonic stem cells and making of chimera rats.
PLoS ONE (Ueda S, Kawamata M, Teratani T, Shimizu T, Tamai Y, Ogawa H, Hayashi K, Tsuda H, Ochiya T.) vol. 3, 2008
33. A comparative analysis of the transcriptome and signal pathways in hepatic differentiation of human adipose mesenchymal stem cells.
FEBS J (Yamamoto Y, Banas A, Murata S, Ishikawa M, Lim CR, Teratani T, Hatada I, Matsubara K, Kato T, Ochiya T.) vol.275, 1260-1273 2008
34. Tissue array substratum composed of histological sections: a new platform for orienting differentiation of embryonic stem cells towards hepatic lineage.
Tissue Eng Part A (Takeuchi T, Ochiya T, Takezawa T.) vol.14, 267-274 2008
35. Identification of erythropoietin-induced microRNAs in haematopoietic cells during erythroid differentiation.
Br J Haematol (Kosaka N, Sugiura K, Yamamoto Y, Yoshioka Y, Miyazaki H, Komatsu N, Ochiya T, Kato T.) vol.142, 293-300 2008
36. Presentation of functional foreign peptides on the surface of SV40 virus-like particles.
J Biotechnol (Takahashi R, Kanesashi S, Inoue T, Enomoto T, Kawano M, Tsukamoto H, Takeshita F, Imai T, Ochiya T, Kataoka K, Yamaguchi Y, Handa H.) vol.135, 385-392 2008
37. RPN2 gene confers docetaxel resistance in breast cancer.
Nat Med (Honma K, Iwao-Koizumi K, Takeshita F, Yamamoto Y, Yoshida T, Nishio K, Nagahara S, Kato K, Ochiya T.) vol.14 No.9, 939-948 2008